IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Court et al.
Application No. 10/692,553
Filed: October 23, 2003
Confirmation No. 1179
For: ENHANCED HOMOLOGOUS RECOMBINATION MEDIATED BY LAMBDA RECOMBINATION PROTEINS
Examiner: Jennifer Ann Dunston
Art Unit: 1636
Attorney Reference No. 4239-66898-01

SUBMITTED VIA EFS ON

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COMMISSIONER FOR PATENTS

DECLARATION OF DR. LIU UNDER 37 C.F.R. § 1.132

1. I, Pentao Liu, Ph.D., am an inventor of the above-referenced application.

2. It is my understanding that claims 1, 3-10, 12, 13, 22 and 23 were rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Cassanova et al. (Genesis 32: 158-160, February 13, 2002) in combination with additional references.

3. A Declaration Under 37 C.F.R. § 1.131, signed by all of the inventors, is of record in the above referenced application. The Office action alleges that the Declaration does not provide sufficient evidence that a nucleic acid encoding a selectable marker flanked by a second pair of recombining sites and a first recombining site was used for homologous recombination. The Office action alleges that the vectors presented in the declaration only include a single pair of LoxP sites flanked by a selectable marker and that the vectors lack the required first recombining site in combination with the second recombining site, as required in claim 1. However, the results presented on pages 9 of the attached laboratory notes (described in the Declaration Under 37 C.F.R. §1.131 on page 4) describe the introduction of the second pair of recombining sites flanking a second selectable marker into a vector that already includes a first pair of recombining sites (in a nucleic acid sequence that can encode a protein once recombination occurs between

these first recombining sites). The results presented on page 10 of the laboratory notes show the excision of the nucleic acid encoding the selectable marker with a second recombinase, which is specific for this second set of recombining sites. After this excision, the first pair of recombining sites (that were introduced previously) remain in the gene. The recombination of the two first recombining sites will produce a nucleic acid that encodes a functional protein. This plasmid is called PL443. Note that the first pair of recombining sites and the second pair of recombining sites are both LoxP sites. Lee et al. (Genomic 73: 56-65, 2001, of record) shows the application of frt/Flp in *E. Coli* based recombineering, see for example Figure 1 of Lee et al. Dr. Lee is an inventor of the present application. Thus, the Declaration under 37 C.F.R. § 1.131 documents that method for generating vectors that utilizes a first and a second pair of recombining sites had been performed prior to February 13, 2002. The Lee publication documents that is was known that frt sites were recombining sites, and documents the creating of a bacterial strain encoding Flp. Thus, it is clear that we conceived of using Frt sites as well, as stated on page 4 of the Declaration under 37 C.F.R. § 1.131.

4. In support of my statement above, additional data is submitted herewith documenting that the vectors my co-inventors and I produced using the claimed methods were introduced into ES cells in the United States prior to February 13, 2002. The PL443 plasmid (created as described above, see also the Declaration Under 37 C.F.R. § 1.131) was linearized with restriction enzyme NotI. The linearized plasmid DNA was electroporated into mouse embryonic stem cells that were subsequently selected in G418 for transfectants. The G418 resistant colonies were picked into 96-well plates for expansion. DNA samples were prepared from these ES cells. These DNA samples were digested with a restriction enzyme and were run on an agarose gel. DNA was then transferred to a nylon membrane using a Southern blotting protocol. The 5' DNA probe was radioactively labeled with ^{32}P and hybridized to the DNA-bound nylon membrane. The hybridized membrane was washed to remove non-specific radioactive signals and was put into a cassette together with an X-ray film for exposure.

The correctly targeted clones (homologous recombination) were clearly visible on the film, a digital image of which is attached. In this image, two bands can be seen, and 18.1kb wild type band and 8.2kb targeted band. Some of them were marked by small checks. This result demonstrated that PL443, a targeting vector made with the design and the recombineering

protocol, functioned well not only in *E. coli* as shown in previous filing, but worked very efficiently in mouse ES cells (up to 70% targeting frequency).

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of the Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Pentao Liu, Ph.D.

March 7th, 2008
Date